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ORIGINAL PAPER

Malathion-induced Oxidative Stress in Rat Brain Regions

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Abstract Malathion is a pesticide with high potential for human exposure. However, it is possible that during the malathion metabolism, there is generation of reactive oxygen species (ROS) and malathion may produce oxidative stress in intoxicated rats. The present study was therefore undertaken to determine malathion-induced lipid peroxidation (LPO), protein carbonylation and to determine whether malathion intoxication alters the antioxidant system in brain rats. Malathion was administered intraperitoneally in the acute and chronic protocols in the doses of 25, 50, 100 and 150 mg malathion/kg. The results showed that LPO in brain increased in both protocols. The increased oxidative stress resulted in an increased in the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), observed in cortex, striatum in the acute malathion protocol and hippocampus in the chronic malathion protocol. Our results demonstrated that malathion induced oxidative stress and modulated SOD and CAT activity in selective brain regions.

Keywords Catalase · lipid peroxidation · malathion · oxidative stress · protein carbonyl · superoxide dismutase

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Introduction

Organophosphorus (OP) compounds are primarily recognized for their neurotoxic effects in mammals through inhibition of acetylcholinesterase (AChE), leading to accumulation of acetylcholine [1]. Recent evidences also suggests that OP compounds may exert direct actions on muscarinic and nicotinic receptors, when their concentration in the circulation exceeds the micromolar level [1, 2].

Malathion [*S*-(1,2-dicarbethoxy)ethyl *O,O*-dimethylphosphorodithioate] is one of the most widely used OP pesticides for agriculture and public health programmes [3]. Malathion is known to induce excitotoxicity through its bioactivated analog, malaoxon [1]. Like other OP compounds, malathion is known to inhibit acetylcholinesterase (AChE) activity, an effect that is thought to underlie the neurotoxicity elicited by these compounds [4]. However, some studies indicate that other biochemical targets may be affected by OP insecticides [5, 6]. Moreover, the lipophilic nature of OPs facilitates their interaction with the cell membrane and leads to perturbations of the phospholipid bilayer structure [7]. The toxicity of OP agents may be due, at least in part, to the formation of reactive oxygen species (ROS), leading to lipid peroxidation, which is generally assessed by an increase in the levels of thiobarbituric acid reactive substances (TBARS) [8–10]. However, there is no report in the literature that describes the effects of malathion exposure in several different rat brain regions after acute or chronic intoxication. Therefore, the present investigation was undertaken to determine oxidative stress and antioxidant response in discrete brain regions of rats following acute or chronic malathion exposure.

Experimental procedure

In vivo studies were performed in accordance with National Institutes of Health guidelines and with the approval of the ethics committee of Universidade do Extremo Sul Catarinense, Criciúma, SC, Brasil.

Animals

Male Wistar rats (age, 2–3 months; weight, 250–320 g) were used in this study. They were housed five to a cage with food and water available *ad libitum*, and were maintained on a 12 h light/dark cycle (lights on at 7.00 a.m.) in a temperature controlled (22°C) colony room. These conditions were maintained constant throughout the experiments.

Treatment

Four doses of malathion (25, 50, 100 and 150 mg/kg/day), dissolved in saline solution (0.9% (w/v) NaCl), were administered intraperitoneally (i.p.) in two treatments. (i) For the acute treatment (n=6 each group), a single injection of malathion was administered. (ii) For the chronic treatment (n=6 each group), malathion was administered daily for 28 days. All control rats received injections of saline solution (0.9% NaCl). These doses were far below the LD_{50} for malathion and caused no overt signs of cholinergic toxicity.

Oxidative stress analyses

Wistar rats were killed by decapitation 24 h after the last malathion administration in the chronic protocol. In the acute protocol animals were killed 1, 6 and 24 h after malathion administration. The brains were removed from the skulls and the cortex, striatum, hippocampus and cerebellum were dissected, and immediately stored at −80°C for biochemical analyses. All the results were normalized by the protein content [11].

As an index of oxidative damage we used the concentration of TBARS and protein carbonyls. To the determination of TBARS the homogenates were mixed with 1 ml of 10% (w/v) trichloroacetic acid and 1 ml of 0.67% (w/v) thiobarbituric acid, then heated in a boiling water bath for 15 min. TBARS were determined by the absorbance at 535 nm [12]. The determination of carbonyl groups was based on the reaction with dinitrophenylhydrazine (DNPH) as previously described [13]. Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in DNPH and the absorbance read at 370 nm.

Antioxidant enzyme assays were performed in tissues homogenates as previously described. To determine catalase (CAT) activity, tissue homogenates were sonicated in 50 mM phosphate buffer and the resulting suspension was centrifuged at 3000×g for 10 min. The supernatant was used for enzyme assay. CAT activity was assayed by measuring the rate of decrease in H_2O_2 absorbance at 240 nm [14]. Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of adrenaline autooxidation, as previously described [15].

Reagents

Thiobarbituric acid, catalase, superoxide dismutase, dinitrophenylhydrazine, adrenaline and hydrogen peroxide were purchased from Sigma, St. Louis, MO, USA.

Statistical analysis

Results are expressed as means ± SD. P-values were considered significant when $P < 0.05$. Differences in experimental groups were determined by one-way analysis of variance (ANOVA). Comparison between means was carried out using a Newman–Keuls test.

Results

Measurement of TBARS

After acute malathion treatment we demonstrated an increase in TBARS as early as 6 h after malathion exposure in the hippocampus and striatum in the higher dose (150 mg/kg) (Fig. 1A). These findings persisted until 24 h after malathion in the cortex (25 mg/kg), striatum (150 mg/kg), cerebellum (all doses) and hippocampus (25 mg/kg) (Fig. 1B). TBARS levels did not change in all doses tested 1 h after acute malathion treatment (data not shown). Surprising, malathion-induced TBARS formation was not dose-dependent.

TBARS concentration in the chronic malathion treatment was significantly increased in the striatum (25 and 150 mg/kg), cerebellum (100 and 150 mg/kg), hippocampus (all doses), but not in the cortex which appeared not to be influenced by chronic malathion treatment (Fig. 1C). These effects were not dose-dependent.

Measurement of protein carbonyls

In contrast with the effects of malathion on TBARs levels, protein carbonyl levels were increased in the cerebellum and cortex 6 h after acute malathion administration (Fig. 2A). After 24 h of acute malathion administration,

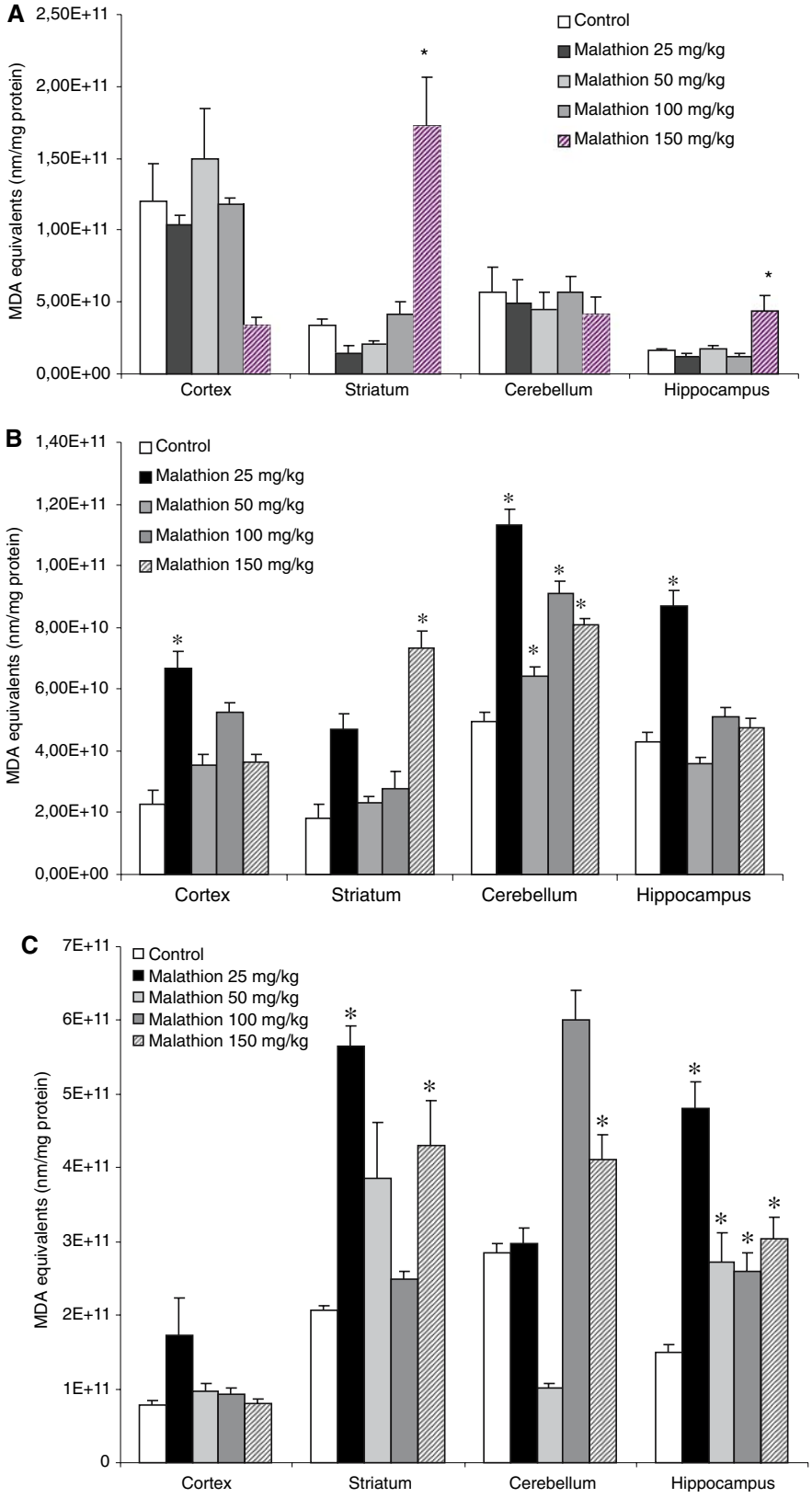

Fig. 1 (**A**, **B**, **C**) Effect of malathion exposure on TBARS content in different brain regions in Wistar rats. Four doses of malathion (25, 50, 100 and 150 mg/kg), dissolved in saline solution (0.9% NaCl), were administered through intraperitoneally injection (i.p.). In the acute malathion treatment rats were killed 1 h (data not shown), 6 h (**A**) and 24 h (**B**) after malathion administration. In the chronic malathion treatment rats were killed 24 h after the last malathion administration (**C**). Cortex, cerebellum, striatum and hippocampus were isolated and TBARS were quantified after treatments as described in Materials and Methods. Values are expressed as mean ± SD of six animals in each group. * Different from control, $P < 0.05$

oxidative damage to proteins was noted only in the cortex. (Fig. 2B). However, the protein carbonyl content in the striatum (25, 50 and 100 mg/kg), cerebellum (25, 50 and 150 mg/kg) and hippocampus (50 and 150 mg/kg) was significantly decreased 24 h after acute malathion treatment (Fig. 2B).

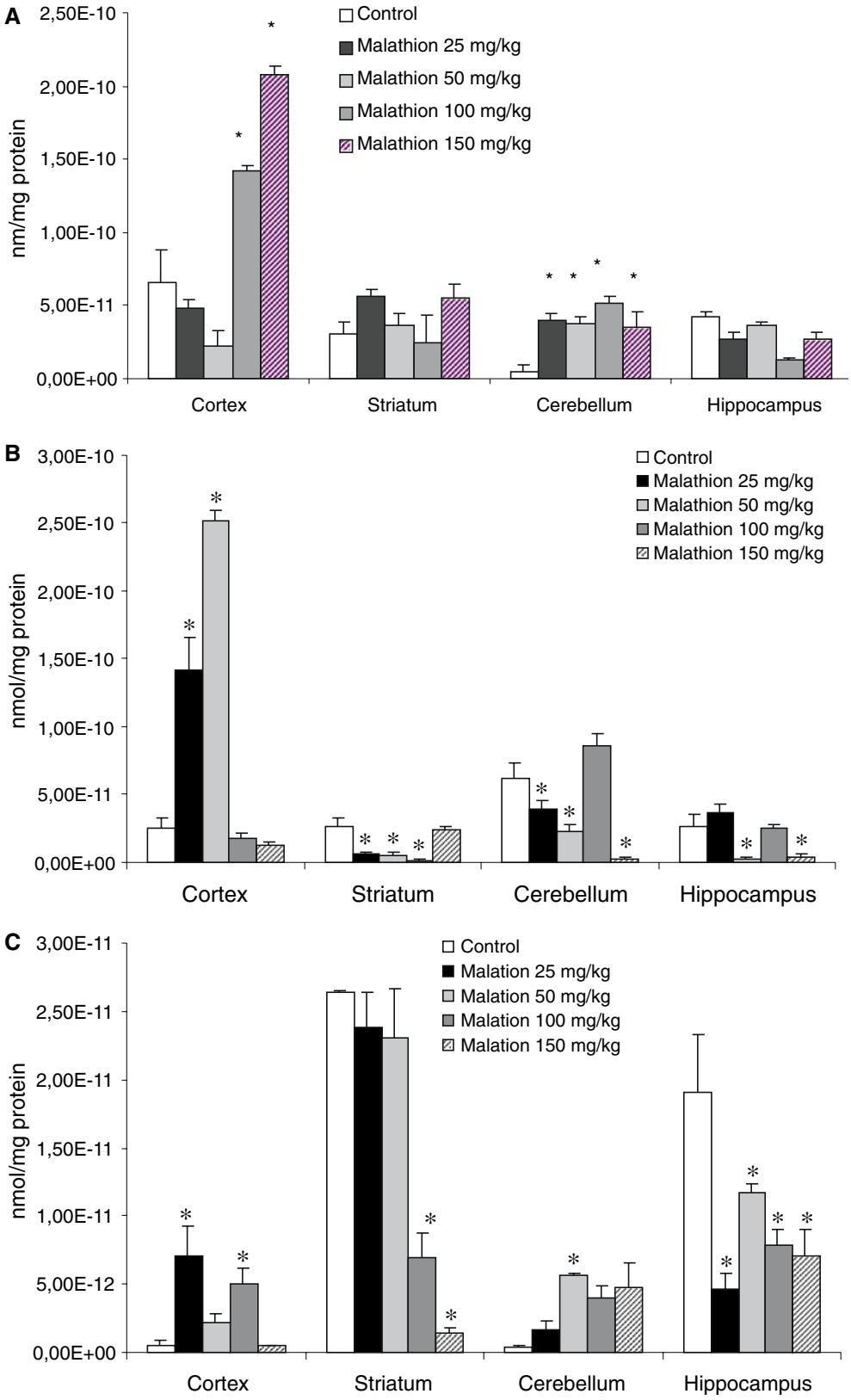

Fig. 2 (**A**, **B**, **C**) Effect of malathion exposure on protein carbonyls content in different brain regions in Wistar rats. Four doses of malathion (25, 50, 100 and 150 mg/kg), dissolved in saline solution (0.9% NaCl), were administered through intraperitoneally injection (i.p.). In the acute malathion treatment rats were killed 1 h (data not shown), 6 h (**A**) and 24 h (**B**) after malathion administration. In the chronic malathion treatment rats were killed 24 h the last after malathion administration (**C**). Cortex, cerebellum, striatum and hippocampus were isolated and protein carbonyls were quantified after treatments as described in Materials and Methods. Values are expressed as mean ± SD of six animals in each group. * Different from control, $P < 0.05$

In the chronic malathion treatment the levels of protein carbonyls were increased in the cortex (25 and 100 mg/kg) and cerebellum (50 mg/kg), but not in the striatum (100 and 150 mg/kg) and hippocampus (all doses) (Fig. 2B). Similarly to the acute protocol the induction of protein carbonyls was not dose-dependent.

Measurement of catalase (CAT) activity

CAT activity did not change 1 h after acute malahtion treatment in all studied structures (data not shown). After 6 h, CAT activity increased in the cortex (25 and 50 mg/kg), cerebellum (25, 50 and 100 mg/kg) and hippocampus (25 and 150 mg/kg) (Fig. 3A). In contrast, 24 h after acute malathion a decreased CAT activity was demonstrated in the striatum (100 and 150 mg/kg) and hippocampus (all doses). In addition, we demonstrated an increased CAT activity in the striatum (25 mg/kg) and cerebellum (50, 100 and 150 mg/kg) (Fig. 3B).

Chronic malathion treatment induced a decrease in CAT activity in the cortex (all doses) and cerebellum (25, 50 and 100 mg/kg). CAT activity increased in the cerebellum (150 mg/kg) and in the hippocampus (25 and 250 mg/kg) 24 h after malathion treatment (Fig. 3B). As demonstrated to the oxidative damage parameters CAT activity alterations were not dose-dependent.

Measurement of superoxide dismutase (SOD) activity

After 1 and 6 h of acute malathion treatment SOD activity was not changed in all regions studied (data not shown). SOD activity was increased 24 h after acute malathion treatment in the cortex (50 mg/kg), striatum (50, 100 and 150 mg/kg), cerebellum (50 and 150 mg/kg) and hippocampus (50 mg/kg). In contrast, SOD activity in the cerebellum (25 mg/kg) and hippocampus (25, 100 and 150 mg/kg) was significantly decreased 24 h after acute malathion treatment (Fig. 4A).

Chronic malathion treatment decreased SOD activity in the cortex (50, 100 and 150 mg/kg), striatum (50 mg/kg), cerebellum (25, 50 and 100 mg/kg) and hippocampus (25 mg/kg). We only observed increased SOD activity in the hippocampus (50 mg/kg) (Fig. 4B). As demonstrated to all other parameters there was no evident dose-dependent response to the alterations in SOD activity after malathion.

Discussion

In this study we determined, for the first time, oxidative parameters in different brain regions after acute or chronic malathion intoxication in rats. Oxidative damage (assessed by the TBARS and protein carbonyls levels) occurs depending on the region analyzed, and, surprisingly, we did not detect a dose-dependence in malathion-induced oxidative stress. These interesting findings suggest that even low malathion doses could induce oxidative damage and these could be related to clinical signs of malathion toxicity. To investigate the impact of malathion on antioxidant defenses in the CNS antioxidant enzyme activity (CAT and SOD) were measured in rats intoxicated with malathion. Overall SOD and CAT activities were not modified in a predictable way, depending on the presence or not of oxidative damage.

Several studies indicate that malathion exposure increases TBARS levels in the erythrocytes, liver, and brain of rats [1, 8]. Other studies indicate that antioxidant enzyme activity was increased, reduced, or not changed in the liver, brain, and erythrocytes of animals treated with this OP compound [1, 3, 8, 16, 17]. The differences found may be due, e.g. to the malathion exposure regime employed, the tissue distribution, or the age of the animals. We demonstrate for the first time that acute and chronic malathion induces an increase in TBARS, but not protein carbonyl, concentration. This suggested increased peroxidation of lipid membranes [8, 18]. Lipoproteins in the central nervous system (CNS) are thought to be essential for lipid transport in the brain but the exact mechanisms of lipid transport and metabolism in the CNS have not been fully characterized [19]. Increased lipid peroxidation was noted in acute malathion poisoning in humans [20].

The basis of OP toxicity in the production of oxidative stress may be due to: (i) Their ‘‘redox-cicling’’ activity (they readily accept an electron to form free radicals and then transfer them to oxygen to generate superoxide anions and hence hydrogen peroxide through dismutation reaction); and (ii) generation of free radicals probably because of the alteration in the normal homeostasis of the body resulting in oxidative stress, if the requirement of continuous antioxidants is not maintained [21]. In addition, organophosphorus insecticides containing the P=S bond (called ‘‘thion’’) are converted to P=O (called ‘‘oxon’’) by a system of enzymes called microsomal mixed-function oxidases (MFO) in which the enzyme cytochrome P-450 plays a major role. The oxons are highly toxic compounds, which account for the profound cytotoxic effects of organophosphorus pesticides. The mutagenic activity of malathion may be due to the existence of electrophillic sites in the parent molecule or its metabolic intermediates, which are capable of binding to nucleophillic sites in biomolecules. In malathion, there are two potential eletrophillic sites—the alkyl group(s) and the phosphoryl group.

Tissues most susceptible to oxidative damage seem to be those with low mitotic rates such as brain that contains easily peroxidizable unsaturated fatty acids and requires very high amounts of oxygen per unit weight (about 20% of the total amount used in humans) [22]. Moreover, in spite of high rate of oxidative metabolism, the brain has relatively low antioxidant defense system [1, 23]. Malathion-induced oxidative damage was not selective to brain regions more prone to oxidative stress such as the dopaminergic pathways (due to the production of hydrogen peroxide during DOPA metabolism) or the nigro-striatal

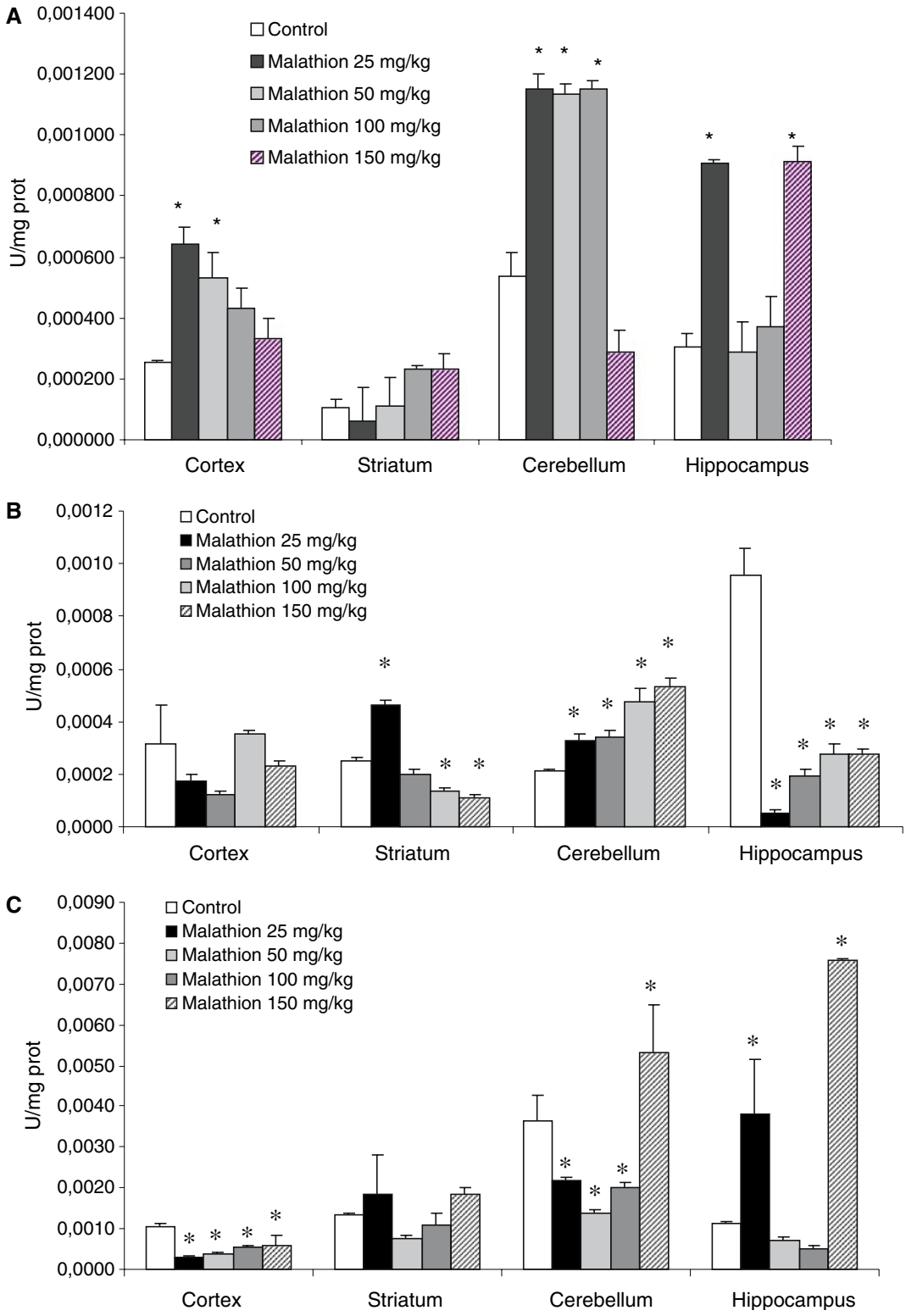

Fig. 3 (**A**, **B**, **C**) Effect of malathion exposure on catalase activity in different brain regions in Wistar rats. Four doses of malathion (25, 50, 100 and 150 mg/kg), dissolved in saline solution (0.9% NaCl), were administered through intraperitoneally injection (i.p.). In the acute malathion treatment rats were killed 1 h (data not shown), 6 h (**A**) and 24 h (**B**) after malathion administration. In the chronic malathion treatment rats were killed 24 h after the last malathion administration (**C**). Cortex, cerebellum, striatum and hippocampus were isolated and catalase activity was quantified after treatments as described in Materials and Methods. Values are expressed as mean ± SD of six animals in each group. * Different from control, $P < 0.05$

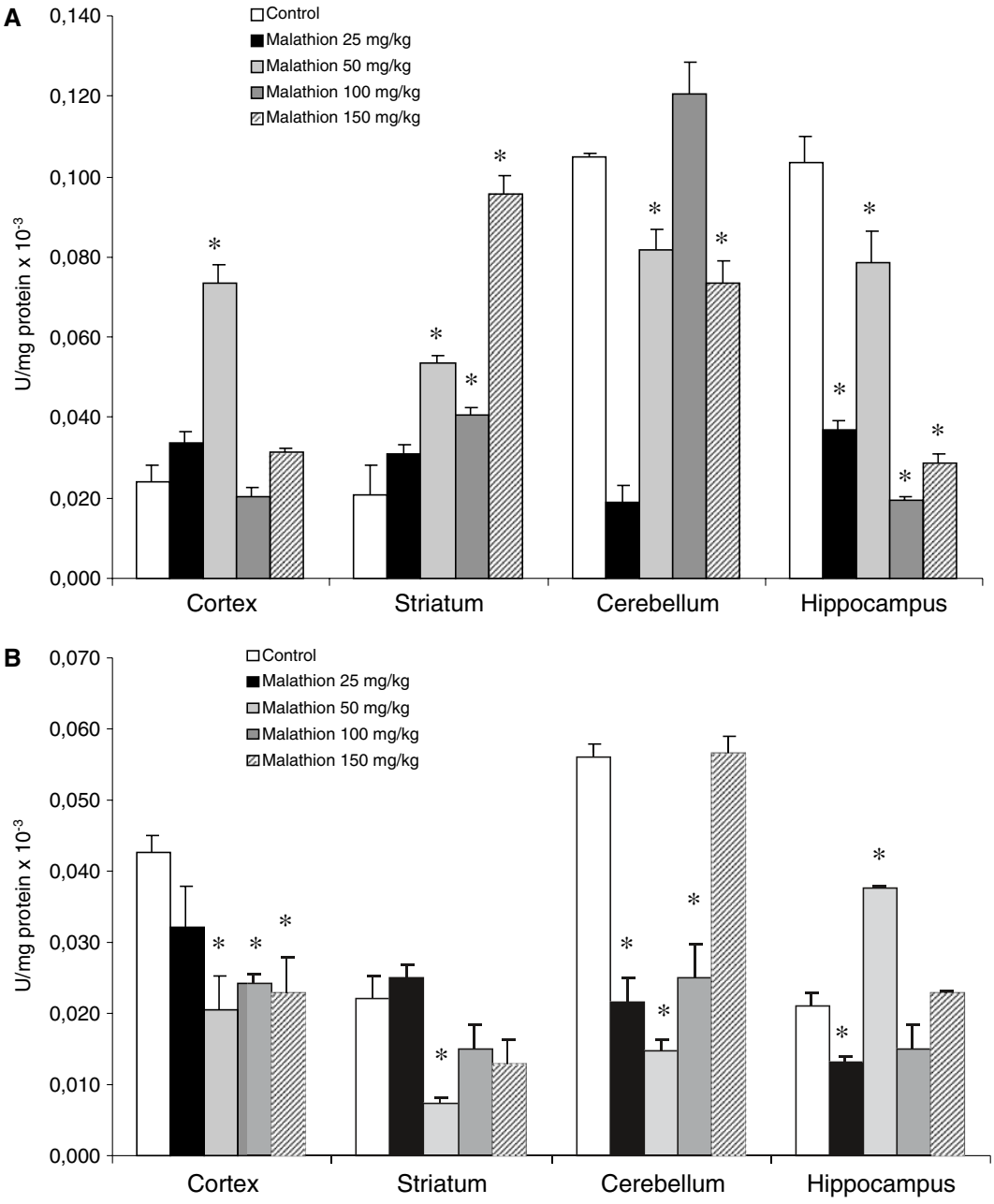

Fig. 4 (**A**, **B**) Effect of malathion exposure on superoxide dismutase activity in different brain regions in Wistar rats. Four doses of malathion (25, 50, 100 and 150 mg/kg), dissolved in saline solution (0.9% NaCl), were administered through intraperitoneally injection (i.p.). In the acute malathion treatment rats were killed 1 h (data not shown), 6 h (data not shown) and 24 h (**A**) after malathion administration. In the chronic malathion treatment rats were killed 24 h after the last malathion administration (**B**). Cortex, cerebellum, striatum and hippocampus were isolated and SOD activity was quantified after treatments as described in Materials and Methods. Values are expressed as mean ± SD of six animals in each group. * Different from control, $P < 0.05$

pathways (due to its high iron content). Carboxylesterases, which are considered to be the main enzymes responsible for the rapid degradation of malathion and malaoxon in mammals, maintain a low activity in brain compared with the major metabolizing organ, liver [1, 16, 24]. This coupled with the greater vulnerability of brain to oxidative stress due to exposure to malathion may, at least partly, explain the maximum amount of MDA production in brain.

Our results suggested that malathion exposure induced oxidative stress and modulated SOD and CAT activities in certain brain regions. The observed oxidative damage could elicit antioxidant response, but published studies

indicate that antioxidant enzyme activity was either increased, reduced, or not changed in the liver, brain, and erythrocytes of animals treated with this OP compound [8, 9, 17]. The differences found may be due, e.g. to the malathion exposure regime employed, the tissue distribution, or the age of the animals. These findings suggested that reactive oxygen species could be involved in the neuronal damage and in the behavioral alterations induced by malathion exposure.

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References

1. Hazarika A, Sarkar SN, Hajare S, Kataria M, Malik JK (2003) Influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. Toxicology 185:1–8.
2. Savolainen K (2001) Understanding the toxic actions of organophosphates. In: Krieger RI (ed) Hanboock of Pesticide Toxicology. Academic Press, New York, p 1013–1041.
3. Ahmed RS, Seth V, Pasha ST, Banerjee BD (2000) Influence of dietary ginger (Zingiber officinales Rosc) on oxidative stress induced by malathion in rats. Food Chem Toxicol 38:443–450.
4. Kwong C (2002) Organophosphate pesticides: biochemistry and clinical toxicology. Ther Drug Monit 24:144–149.
5. Ward R, Mundy WR (1996) Organophosphorus compounds preferentially affect second messenger systems coupled to M2/M4 receptors in rat frontal cortex. Brain Res Bull 39:49–55.
6. Samimi A, Last JA (2001) Mechanism of inhibition of lysyl hydroxylase activity by the organophosphates malathion and malaoxon. Toxicol Appl Pharmacol 176:181–186.
7. Videira RA, Antunes-Madeira MC, Lopes VI, Madeira VM (2001) Changes induced by malathion, methylparathion and parathion on membrane lipid physicochemical properties correlate with their toxicity. Biochim Biophys Acta 1511: 360–368.
8. Akhgari M, Abdollahi M, Kebryaeezadeh A, Hosseini R, Sabzevari O (2003) Biochemical evidence for free radical induced lipid peroxidation as a mechanism for subchronic toxicity of malathion in blood and liver of rats. Human Exp Toxicol 22:205–211.
9. Brocardo PS, Pandolfo P, Takahashi RN, Rodrigues ALS, Dafre AL (2005) Antioxidant defenses and lipid peroxidation in the cerebral cortex and hippocampus following acute exposure to malathion and/or zinc chloride. Toxicology 207:283–291.
10. Abdollahi M, Mostafalou S, Pournourmohammadi S, Shadnia S (2004) Oxidative stress and cholinesterase inhibition in saliva and plasma of rats following subchronic exposure to malathion. Comp Biochem Physiol C Toxicol Pharmacol 137: 29–34.
11. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ (1951) Medida de proteína com o Folin fenol reagente. J Biol Chem 193:265–275.
12. Draper HH, Hadley M (1990) Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 186:421–431.
13. Levine RL, Garland D, Oliver CN (1990) Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol 186:464–478.
14. Dal-Pizzol F, Klamt F, Bernard EA, Benfato MS, Moreira JCF (2001) Retinol supplementation induces oxidative stress and modulates antioxidant enzyme activities in rat Sertoli cells. Free Radic Res 34:395–404.
15. Bannister JV, Calaberese L (1987) Assays for SOD. Methods Biochem Anal 32: 279–312.
16. Srikanth NS, Seth PK (1990) Alteration in xenobiotic metabolizing enzymes in brain and liver of rats coexposed to endosulfan and malathion. J Appl Toxicol 21:333–337.
17. John S, Kale M, Rathore N, Bhatnagar D (2001) Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erytrocytes. J Nutr Biochem 12:500–504.
18. Vidella LA, Baros SBM, Junqueira VBC (1990) Lindane induced liver oxidative stress. Free Radic Biol Med 9:169–179.
19. Kock S., Beisiegel U. Lipoproteins in the brain: A new frontier? In: Betteridge DJ (ed) Lipids and Vascular Disease, Martin Dinitz, London, pp 51–64.
20. Banerjee BD, Seth V, Bharracharya A, Pasha ST, Chakraborty AK (1999) Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers. Toxicol Lett 107:33–47.
21. Vidyasagar J, Karunakar N, Reddy MS, Rajnarayana K, Surender T, Krishna DR (2004) Oxidative stress and antioxidant status in acute organophosphorus insecticide poisoning. Indian J Pharmacol 36:76–79.
22. Floyd RA, Hensley K (2002) Oxidative stress in brain aging implications for therapeutics of neurodegenerative diseases. Neurobiol Aging 23:795–807.
23. Mates JM (2000) Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology 153:83–104.
24. Talcott RE (1979) Hepatic and extrahepatic malathion Carboxylesterases. Assay and localization in the rat. Toxicology 47:145–150.